Biodegradable PBAT/PLA blend with bioactive MCPA-PHBV conjugate suppresses weed growth

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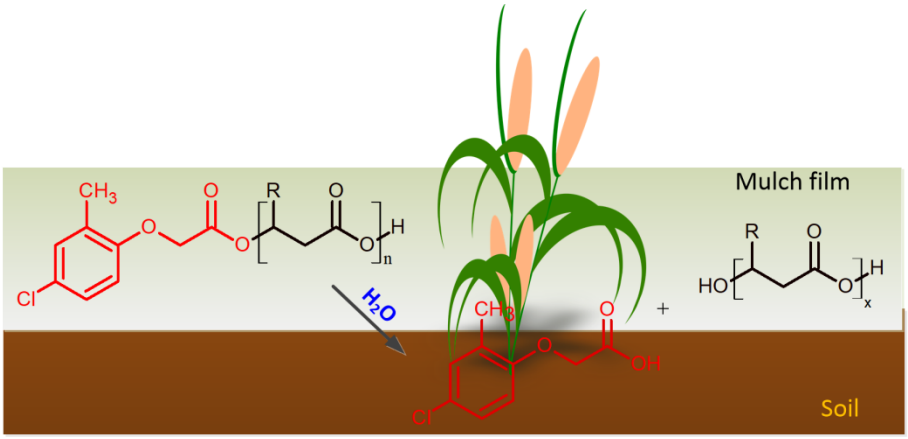

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KEYWORDS

MCPA conjugate; polyhydroxyalkanoates; biodegradability; PBAT; PLA, mulch

ABSTRACT

The herbicide 2-methyl-4-chlorophenoxyacetic acid (MCPA) conjugated with poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV) was prepared via a melt transesterification route. The resultant bioactive oligomer was then mixed with a blend of polylactide (PLA) and poly(butylene adipate-*co*-terephthalate) (PBAT) with different loadings; to manufacture films to be used as a bioactive, biodegradable mulch to deliver the herbicide to target broadleaf weed species. The biological targeting of the MCPA-PHBV conjugate in the mulch film was investigated under glasshouse conditions using faba bean (*Vicia faba*) as a selective (non-target) model crop species having broadleaf morphology. The presence of the MCPA-PHBV conjugate in the biodegradable PBTA/PLA blend was shown to completely suppress the growth of broadleaf weed species, whilst displaying only a mild effect on the growth of the model crop. The degradation of the mulch film under glasshouse conditions was quite slow. The release of the MCPA-PHBV during this process was detected using NMR, GPC, EDS and DSC analyses, indicating that the majority of the MCPA diffused out after MCPA-PHBV conjugate bond scission. These data provide a strong "proof of concept" and show that this biodegradable, bioactive film is a good candidate for future field applications and may be of wide agricultural applicability.

INTRODUCTION

Agrochemicals are commonly used in agriculture to ensure high crop yield. However, because of their low resistance to environmental conditions, a sizeable proportion of these chemical compounds do not reach their target, which subsequently results in environmental pollution and low efficiency. This is because the bioactive compounds are rapidly broken down in the environment before they can be absorbed by the plants, most often due to surface run-off by heavy rainfall or other factors during the growing season. The herbicide 4-chloro-2-methylphenoxyacetic acid (MCPA) is widely used to control annual and perennial broad leaved weeds, including poppy, thistles and docks, in crops such as cereals, rice, linseed, flax, grassland and turf. However, MCPA has been included on the list of priority pollutants of the European Union and the US Environmental Protection Agency (EPA).[1] Therefore, it is necessary to develop effective management practices to control environmental contamination by MCPA.

One of the ways to reduce these adverse effects is *via* the use of "controlled release" systems, which gradually release the bioactive compounds over a prolonged period of time.[2] Various carriers have been used for these bioactive substances such as calcium alginate,[3] hydroxyapatite,[4] and biodegradable polymers.[5-7] Traditionally, the bioactive substance is simply admixed with the carrier, which will retard its loss by volatilization and leaching.[5] In the case of biodegradable polymers, the agrochemicals may be carried in the polymers in different forms such as nano- and microparticles, and the release of the chemicals is usually *via* the following three mechanisms: diffusion through pores, diffusion through the polymer matrix, osmotic pumping and erosion.[8]

The transesterification of polyhydroxyalkanoates (PHA) has been used as a tool for the preparation of delivery systems containing covalently bonded MCPA.[9] Recently, we reported the synthesis of pesticide-oligomer conjugates, namely the conjugates of MCPA or (2,4-

dichlorophenoxy) acetic acid (2,4-D) with atactic oligo(3-hydroxybutyrate) *via* anionic ring-opening oligomerization of β-butyrolactone initiated by the salt of selected pesticides[10]. The effectiveness of the resultant pesticide-oligomer conjugates was confirmed by field bioassays.[11] Furthermore, the synthetic approach for the biodegradable release systems based on anionic homo- and copolymerization of respective β-substituted β-lactones containing MCPA moieties was reported.[12] However, the alternative approach of producing PHA-MCPA conjugates via the transesterification of natural PHA, would seem to be more pragmatic, from both a technological and economic point of view.

In the current study, we investigated the delivery of MCPA *via* the use of biodegradable mulch films. Biodegradable mulch as a sustainable alternative to polyethylene (PE) mulch has been used on a limited scale in many agricultural systems since the late 1990s, and shows significant advantages for plant growth in terms of both root length and lateral root mass density[13]. Recently, the European Parliament voted in favour of supporting biodegradable mulch films in the revision of the EU Fertilizers Regulation.[14] In theory, embedding herbicide into such biodegradable mulch would facilitate both a slow release of the herbicide, whilst also functioning as a mulch film.

The blending of a small proportion of herbicide directly with biodegradable polymers often results in processing problems. In our study, the active compound is covalently bound to the polymer by a labile bond at the end of a poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV) oligomer.[9] Release depends upon the rate of chemical or biological cleavage of the PHBV oligomer-MCPA bonds. Our hypothesis is that after the oligomer-MCPA bond cleavage, the herbicide can migrate into the soil *via* diffusion through the mulch to suppress the growth of target weed species. A blend of polylactide (PLA) and poly (butylene adipate-*co*-terephthalate

(PBAT) is used as the model biodegradable polymer for manufacturing the mulch. PBAT/PLA blends have been widely investigated for their biodegradability and have been used to produce viable mulch films in numerous studies.[15-19]

The objective of the work presented was to determine the degradation and the release of the herbicide from the mulch film under glasshouse conditions and to establish its efficacy to supress broadleaf weed growth without significant phytotoxicity to crop species. The results of which, would help inform the design and synthesis of conjugates with optimal properties and concentrations for commercial use in high value crop systems.

MATERIALS AND METHODS

Materials. The polyester blend of poly(butylene adipate-*co*-terephthalate) (PBAT) and polylactic acid (PLA), under the commercial name BioFlex, was kindly provided by FKuR Kunststoff GmbH (Germany). According to ^{1}H NMR analysis, the blend contained 30 mol% of PLA and 70 mol% of PBAT, and the latter contained 47 mol% of aromatic segments. The MCPA used in this work was purchased from Sigma-Aldrich (UK) and was used as received. The PHBV was purchased from Tianjin Green Bio-Science (Tianjin, China). According to ^{1}H NMR analysis, the content of HV units was below 3 mol% and this was also confirmed by ESI-MS analysis.

Synthesis of the MCPA-PHBV conjugate. The novel MCPA-PHBV conjugate was prepared *via* thermal transesterification. PHBV and MCPA was melt blended in a Brabender mixer at a temperature of 170^{o}C for 10 minutes under the catalysis of p-toluenesulfonic acid monohydrate. The reaction mixture was then purified by being dissolved in chloroform and then precipitated using hexane. The conjugate had a number average molecular mass (M_n) of 800 g/mol.

Film preparation. Mulch films were prepared at Fraunhofer UMSICHT (Germany) using a hot pressing method. Various concentrations of the conjugates were compounded with the polyester matrix on a rolling mill (LRM-SC-110/T3E Scientific Laboratory Two-Roll Mills, Labtech Engineering Co., Ltd) at 140°C and 30 rpm roll speed. The rolling mill was used because only small amounts of material were required. The rolling mill is not suited for preparation of optimal homogeneous compounds. The films were prepared using a hydraulic laboratory press (LP-S-20, Labtech Engineering Co., Ltd) at 160°C and 100 kN. Three samples were prepared containing MCPA-PHBV conjugate at 5, 10 and 15% respectively, and the samples were designated as V6-5%, V6-10% and V6-15%. The control film sample (without bioactive conjugate) V6-0% was prepared according to the same procedure. The film thickness was approximately 200 μm.

Glasshouse application of the mulch film. Glasshouse experiments were designed using a completely randomized factorial design having six replications. Plants were grown in ϕ10 × 40 cm cylidrical plastic pots filled with 3.6 kg top soil collected from Hilton research station (University of Wolverhampton, UK), which has natural weed infestation. Faba bean (*Vicia faba*) inbred line Misr 3 was used as a selective (non-target) crop species in these experiments. Faba bean seeds were germinated on moist Whatman filter paper at 25°C for 72 h in an incubator. Initially, two radicle-emerged seeds were sown at 3 cm depth in each pot on 11th July 2016 and were later thinned to one plant per pot. Two week after planting, the mulch films containing 0, 5, 10 and 15% concentrations of the MCPA-PHBV conjugate were placed over the soil surface while no film was used in the negative control. Plants were grown in a glasshouse located at the University of Wolverhampton. The ambient glasshouse temperature was maintained at 25°C ± 5°C. Supplemental lighting was provided by Phytolux Attis7 LED growth lights to maintain

photosynthetic photon flux density (PPFD) of around 300 μmol m^{-2} s^{-1} at the canopy level. The recording of experimental data on parameters related to plant health were initiated when the plants were four weeks old, and terminated when the plants were seven weeks old. The mulch films were sampled for analysis after four months of plant growth.

Stomatal conductance was determined using a Porometer (SC1 - Decagon, USA) on the abaxial surface of the leaflets of the upper-most fully expanded leaf. Chlorophyll content readings of three leaflets of a fully lit, youngest expanded leaf were taken with a Chlorophyll Meter (SPAD-502 plus, Konica Minolta, Japan) while photosynthetic efficiency was determined with a Chlorophyll Fluorometer (Mini-PAM II Photosynthesis Yield Analyzer, WALZ, Germany). The data recorded during the course of the experiments were subjected to analysis of variance using statistical software IBM SPSS 24 and means were compared using the least significant differences (LSD) at a 5% level of probability ($P< 0.05$).

Scanning electron microscopy (SEM) and Energy-Dispersive X-ray Spectroscopy (EDS). In order to assess the surface structure of the film (with and without MCPA-PHBV conjugate) both before and after the glasshouse experiments, a small sample of each film (approximately 10 × 10 mm) was mounted on an SEM stub, using double-sided carbon sticky tabs (Agar Scientific G3348N). The stub/sample was then coated with approximately 12 nm of Pt in an Emscope SC 500 sputter coater. Samples were then examined in a Hitachi TM3030 SEM at 15kV.

In addition, an elemental distribution scan for the elements present on surface of the samples was performed using energy-dispersive X-ray spectroscopy (EDS) using an Oxford Instruments SwiftED3000 module attached to the SEM. The objective of this analysis was to determine the distribution/quantity of chlorine (a signature element present in the MCPA herbicide, but not in the polymer matrix) both before and after the glasshouse experiments.

Thermal analysis. The thermal properties of the mulch were determined by differential scanning calorimetry (DSC 204 Phoenix, NETZSCH-Gerätebau GmbH, Germany) under a nitrogen atmosphere. The sample weights were around 8 mg for all of the measurements made. Samples were first cooled from 20°C to -50°C with a cooling rate of 20 K/min and held for 4 min. The first heating run was from -50°C to 200°C with a heating rate of 20 K/min and held for 1 min. The cooling run was from 200°C to -70°C with a cooling rate of 20 K/min and held for 4 min again. The second heating run was from -70°C to 200°C with a heating rate of 20 K/min. The glass transition temperature (T_g), crystallization temperature (T_c) and melting temperature (T_m) were determined from the second heating scans. The first cooling was used to determine the actual condition of a sample and to observe the possible thermal aging of the material. The first heating was used to erase the thermal history of the samples.

Nuclear magnetic resonance (NMR). The ^{1}H NMR spectra of the MCPA-PHBV oligomer were recorded on a Bruker-Advance spectrometer, operating at 600 MHz. The ^{1}H NMR spectra of the film during the degradation test was recorded on a JEOL ECX-400 (400 MHz) spectrometer. The solvent for the NMR experiments was chloroform-*d* and tetramethylsilane (TMS) was used as the internal standard.

The PBAT/PLA blend composition was determined based upon integration of the signal of the methine group of the PLA component (at δ = 5.20 ppm) and the signals of methylene groups in aromatic and aliphatic dyads presented in ^{1}H NMR spectrum in the region between δ = 4.0 to 4.5 ppm. The composition of the PBAT component (mol % of aromatic and aliphatic units) was calculated based upon the intensities of the signals of methylene groups in aromatic and aliphatic dyads (in region between δ = 4.0 to 4.5 ppm) according to literature.[18]

Gel permeation chromatography (GPC). The molar mass and molar mass distribution of the MCPA-PHBV oligomer was characterized using a Viscotek VE3580 system. The GPC experiments were conducted in chloroform solution at 35^{o}C at a flow rate of 1 ml/min using a Viscotek VE 1122 solvent delivery system with two Mixed C PL-gel styragel columns in series and a Shodex SE 61 refractive index detector. A volume of 10 µl of sample solutions in chloroform (concentration 0.5 % m/V) was injected into the system. Polystyrene standards with low dispersity were used to generate a calibration curve.

The molar mass and molar mass distribution of the films collected at regular time intervals during the glasshouse tests were analysed using a TOSOH EcoSec HLC/GPC 8320 system equipped with a RI and a UV detector, operating at a temperature of 40^{o}C. The column used was TSKgel HZM-N calibrated against polystyrene standards with low dispersity ranging from 560 to 70000 Da. The UV detector was set at a wavelength of 254 nm. Chloroform was used as the eluent at a flow rate of 0.25 ml/min. A sample size of 2 µl was injected into the system using an autosampler.

Electrospray ionisation mass spectrometry (ESI-MS). ESI-MS analysis was performed using a Finnigan LCQ ion trap mass spectrometer (Thermo Fisher Scientific Inc., San Jose, CA, USA). The chloroform/methanol (1:1 V/V) solutions of samples were introduced to the ESI source by continuous infusion using the instrument syringe pump at a rate of 10 µl/min. The ESI source of the LCQ was operating at 4.5 kV and the capillary heater was set to 200^{o}C. Helium was used as the nebulising gas. The analyses were performed in the positive- and negative-ion mode.

RESULTS AND DISCUSSION

In the current study, a MCPA conjugated PHBV (not previously reported) was mixed with a PBAT/PLA blend to prepare a bioactive and biodegradable mulch film. The use of MCPA in a conjugate form with PHBV facilitates the processing of the mix into a film form. The film also functioned as a mulch for agricultural production, the purpose of which was not to affect the growth and development of non-target crop species (in this case *Vicia faba*). For evaluation of the efficacy of the film, we performed a glasshouse test of the film under controlled conditions.

Structure of MCPA-PHBV oligomer at the molecular level

MCPA-PHBV oligomer was synthesized using a melt transesterification method. After purification as described in the experimental section, the ^{1}H NMR spectrum of the conjugate obtained in the reaction between MCPA and PHBV is presented in Figure 1. Signals corresponding to the protons of the 3-hydroxybutyrate repeating units (signals 1-3) were observed, while signals corresponding to 3-hydroxyvalerate repeating units were not clearly visible in the spectrum due to low content of these units in the initial biopolymers (below 3%). Moreover, signals corresponding to the protons of the pesticide bonded to the oligomers (4-8), as well as the signals of the protons present in the unbonded pesticide (4'-8'), were also observed. Additionally, low-intensity signals corresponding to protons of the crotonate end group were detected (protons of the CH3- at δ =1.87 ppm, vinyl protons, -CH=CH-, at δ = 5.80 ppm and δ = 6.95 ppm).

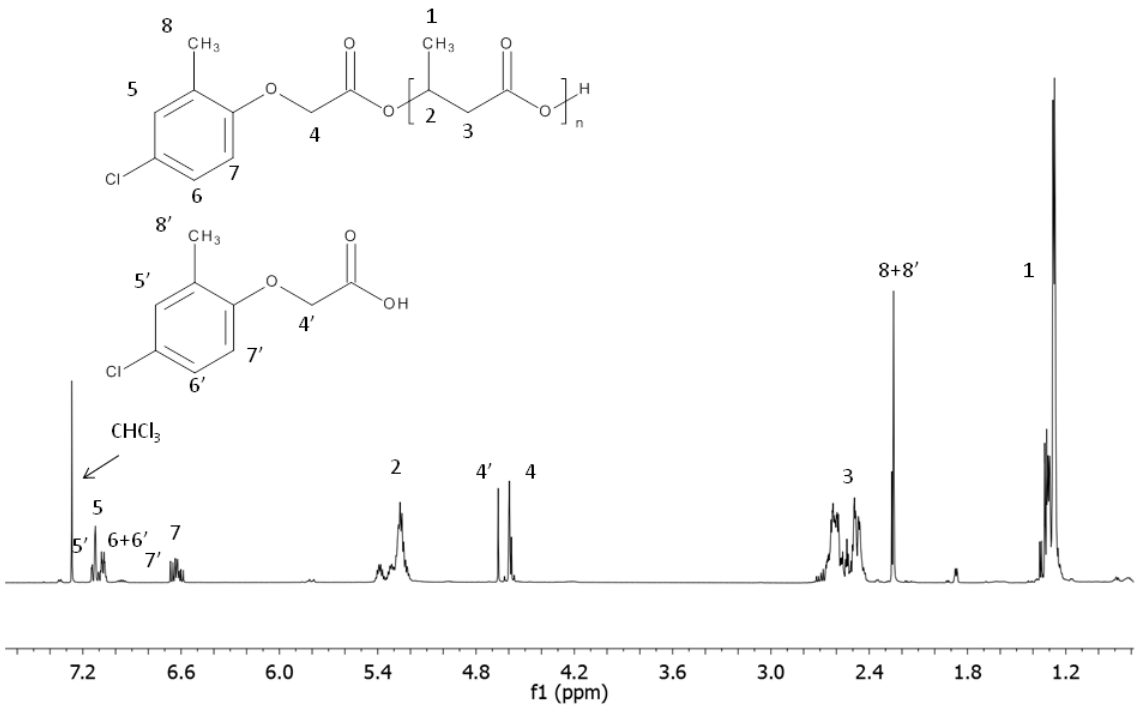

Figure 1. The ^{1}H NMR spectrum of the MCPA-PHBV conjugate prepared using melt blending method in a Brabender mixer at a temperature of 170^{o}C for 10 minutes under the catalysis of p-toluenesulfonic acid monohydrate.

The ESI-MS spectrum of the product obtained in the reaction between MCPA and PHBV is presented in Figure 2. The main series of ions visible in the mass spectrum, labelled as A (calculated according to the formula *m/z* 200+(86×n)+23), corresponds to the sodium adduct of herbicide conjugated with the PHA oligomers. The second series of ions present in ESI-MS spectrum, labelled as B (calculated according to the formula *m/z* 86+(86×n)+23) corresponds to the sodium adduct of oligomers with crotonate and carboxyl end groups. The third series of ions (labelled as C) visible in the spectral expansion in the range m/z 960-1020, corresponds to the sodium adduct of oligomers with hydroxyl and carboxyl end groups (calculated according to the formula m/z 104+(86×n)+23).[9] Despite the low content of HV units, the series of ions labelled as A' visible in the spectral expansion in the range m/z 960-1020, corresponds to the sodium adduct

of herbicide conjugated with the biopolyester cooligomers, contained one HV repeating unit and eight HB units (calculated according to the formula m/z 200+(86×n)+(100×m)+23).

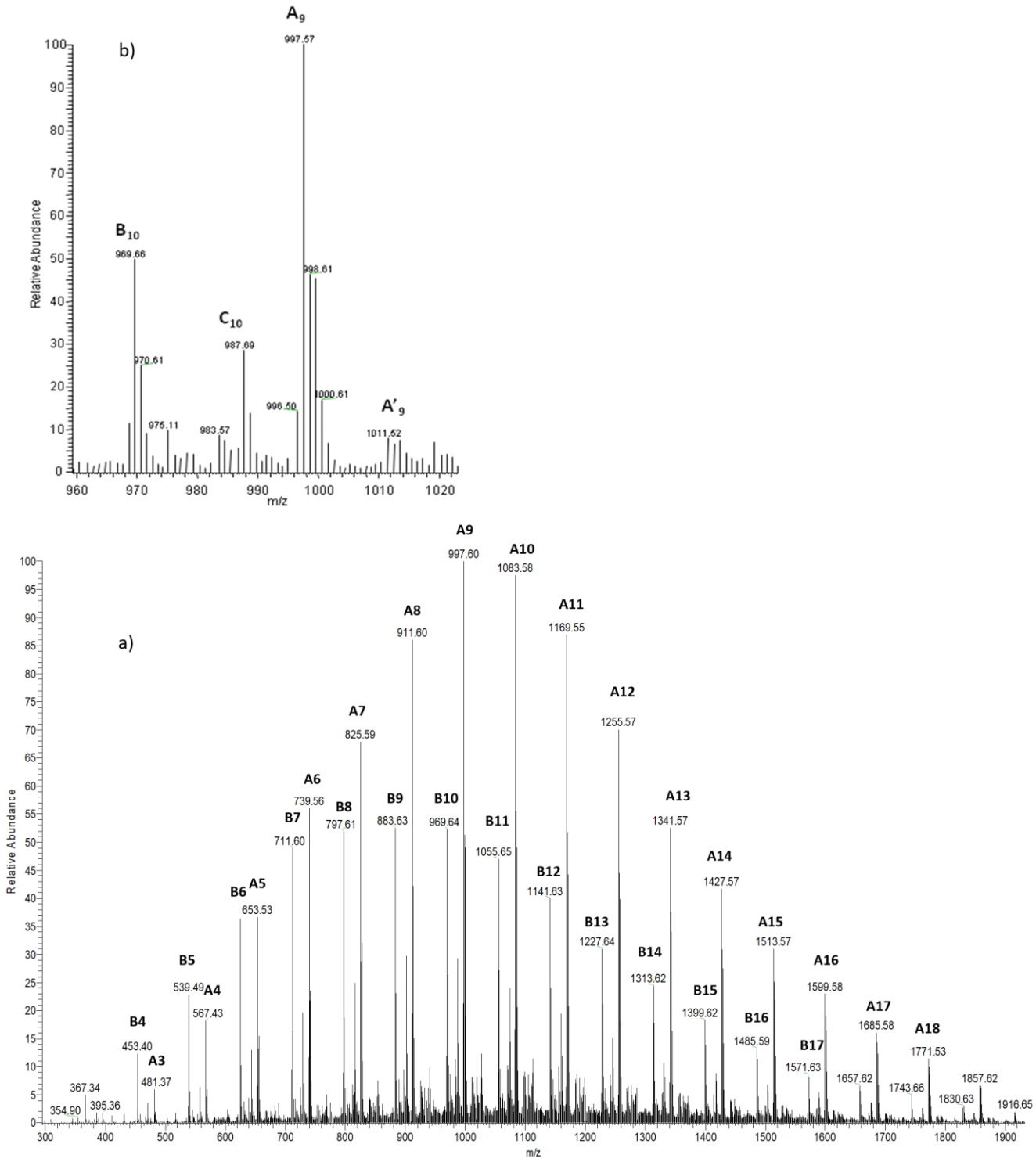

Figure 2. ESI-MS spectrum (in positive-ion mode) of the MCPA-PHBV conjugate prepared using melt blending method in a Brabender mixer at a temperature of 170°C for 10 minutes under the catalysis of *p*-toluenesulfonic acid monohydrate. The spectrum a) is in the range 200-1800, and spectrum b) is in the range m/z 780–1020 to show the C series.

The ESI-MS^2 spectrum of the sodium adduct of MCPA conjugated with the biopolyester oligomers at *m/z* 1083 is presented in Figure 3. The product ions at m/z 997, 911, 825, 739, 653, 567, 481 and 359 correspond to the oligo(3-hydroxybutyrate) terminated by the MCPA and carboxyl end groups. The complementary product ions at m/z 883, 797, 711, 625, 539, 453 and 367 correspond to the oligo(3-hydroxybutyrate) terminated by crotonate and carboxyl end groups. For example, the product ion at m/z 883 corresponds to the oligomer formed by the loss of the MCPA molecule (200 Da), and the product ion at m/z 997 corresponds to the oligomer formed by the loss of crotonic acid (86 Da).

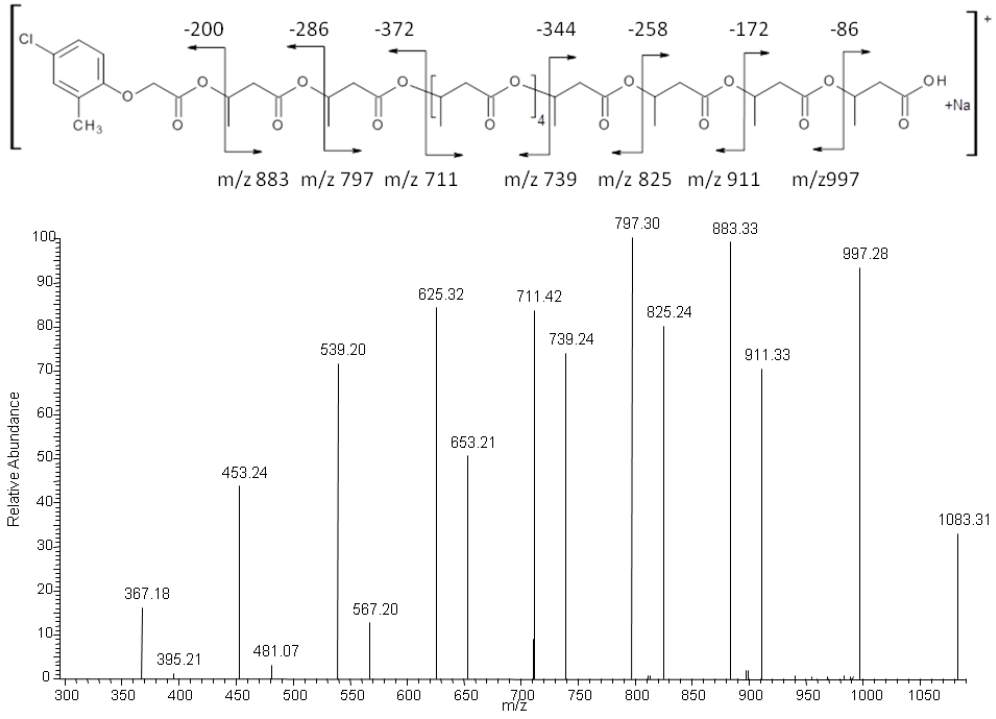

Figure 3. ESI-MS^2 spectrum of the sodium adduct of MCPA conjugated with PHBV oligomers at *m/z* 1083.

Effect of mulch containing MCPA-PHBV on target and non-target plant growth

The two objectives of the glasshouse experiment were to test the effectiveness of the bioactive mulch on weed suppression (broadleaf weed species) and on the growth of the model (non-target) crop species (*Vicia faba*). As shown in figure 4, weed species flourished in the negative controls i.e. when no mulch was applied and when mulch film which did not contain MCPA-PHBV was applied. However, the weed species were clearly suppressed when MCPA-PHBV containing mulch was applied. No weed growth was observed even when the mulch containing the lowest MCPA-PHBV concentration of 5% was used. These data indicate that the MCPA-PHBV embedded into the films was very effective at controlling broadleaf weed growth at all the concentrations tested.

The results of a number of growth parameters which were used to monitor plant health of the non-target, crop species (faba bean) during the course of the glasshouse experiments are shown in Figure 5. Data on yield-II of chlorophyll fluorescence showed non-significant differences ($P< 0.92$) between MCPA-PHBV containing mulch and the negative controls. However, when stomatal characteristics of the faba beans were investigated, the stomatal conductance values showed a declining trend for the MCPA-PHBV containing mulch. The least stomatal conductance (92.6 μmol/(m^2s) was recorded in the V6-10% mulch, which was significantly lower than the negative control minus the mulch (168 μ mol/m^2 /s) and the V6-0% negative control (170 μmol/(m^2s) treatment. Chlorophyll content or greenness of leaves is another commonly used indicator of plant health. The data obtained for the leaf chlorophyll content, suggests that there was observed differences in the SPAD index between the negative controls

and the test plants after applying mulch containing the MCPA-PHBV oligomer. These data demonstrate that the mulch film does not affect photosynthetic efficiency in this "proof of concept" experiment. However, the leaf chlorophyll content of the non-target crop species (*Vicia faba*) produced inconsistent results at higher concentrations of MCPA. Furthermore, the stomatal conductance was negatively correlated with increasing MCPA-PHBV concentration in the mulch film. Further studies to establish the most effective MCPA level, for efficient weed suppression, using a wider range of concentrations, which with produce little or no adverse effect on the plant health of the non-target crop species, is currently underway.

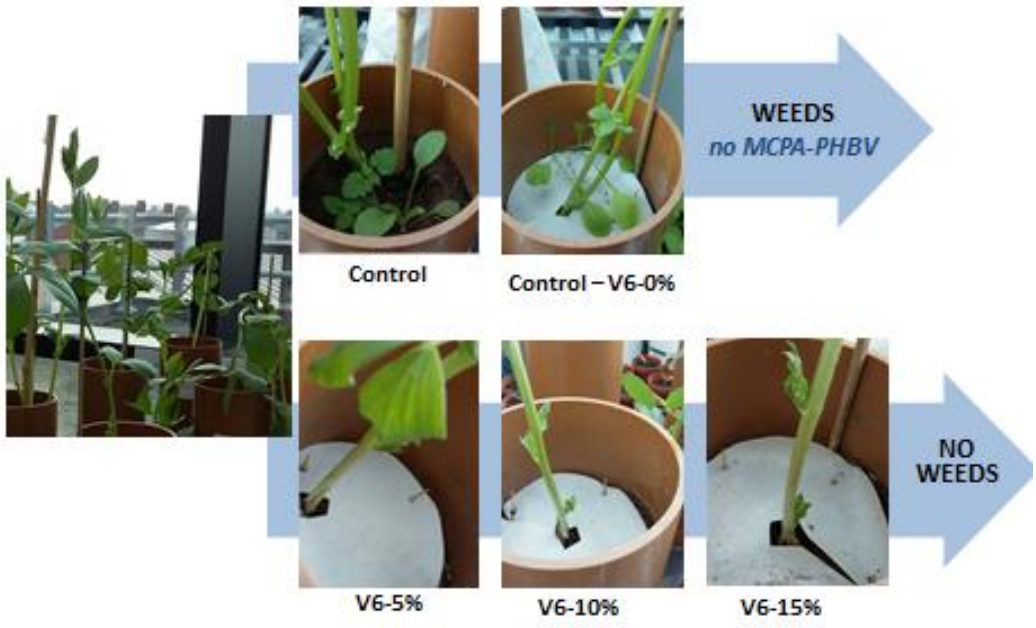

Figure 4. Glasshouse experimental design to test the effect of the bioactive mulch film on the suppression of weed growth and on the health of the model (non-target) crop species (faba bean).

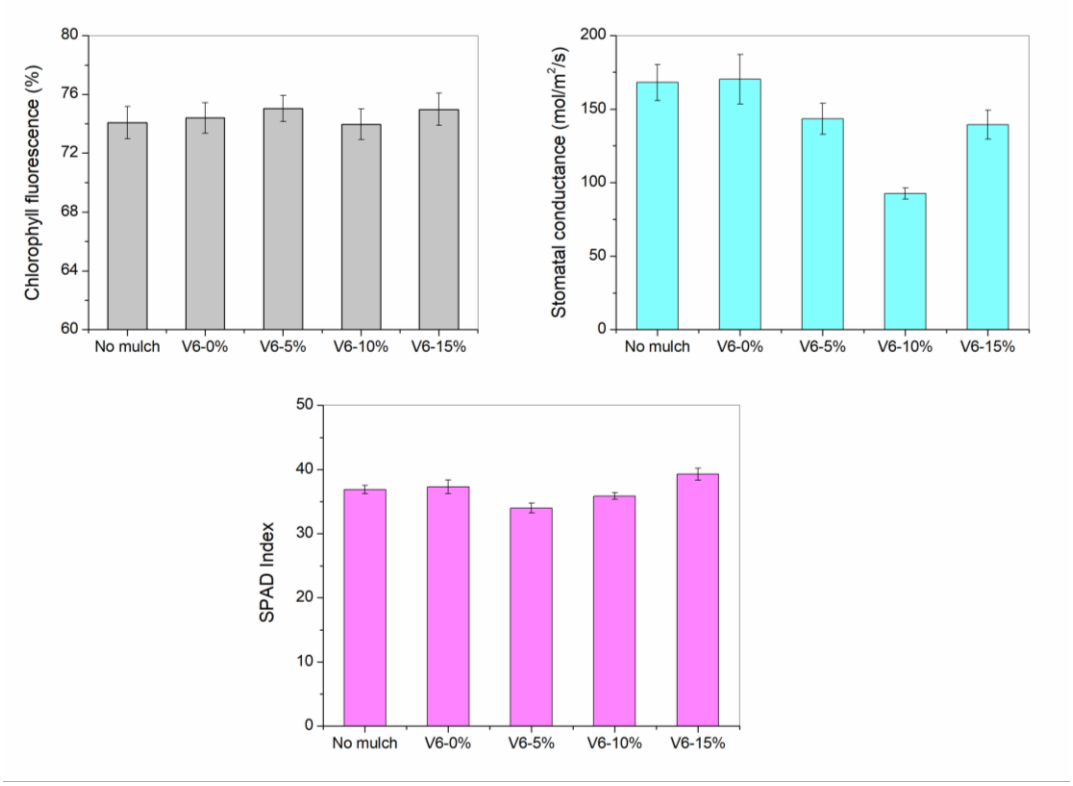

Figure 5. Effect of MCPA-PHBV concentration on the growth parameters of faba bean plants. Three growth parameters (photosynthetic efficiency, stomatal conductance and SPAD index) are plotted against the treatments of MCPA-PHBV concentration in comparison with no mulch as the negative control. The vertical error bars represent standard error based on six replications.

Migration of MCPA into the soil

SEM and EDS analyses of the mulch films. The surface of the mulch films before and after the glasshouse experiments was examined using SEM, the results of which are shown in Figure 6. The surface of the mulch film containing no MCPA-PHBV conjugate prior to the glasshouse experiment were quite smooth, with small ridges which were formed during processing, running diagonally across the surface of the film. The films containing MCPA-PHBV have a rougher surface with numerous ridges and furrows across the surface in a variety of directions across the

film. After 4 months contact with soil, the surface markings on the MCPA-PHBV containing samples became less distinct (Figure 6).

The cause of the rough morphology of the surface of the film containing the MCPA-PHBV is most likely due to the presence of a layer of the conjugate on the surface of the polymer. After four months contact with the soil, the surface of the films containing 5% and 10% MCPA-PHBV became smoother, with morphology quite similar to that of the control sample (without MCPA-PHBV). However, the surface of the film containing 15% MCPA-PHBV still remained rough after exposure to the soil, but over the course of the experiment the degree of roughness was reduced. It can thus be inferred that the roughness is related to the MCPA-PHBV concentration. After four months contact with soil, the surface roughness was reduced due to the gradual removal of the MCPA-PHBV oligomer or the herbicide from the film with the passage of time. The data obtained from the GPC experiment (as described in more detail later) demonstrated that the mulch samples have not degraded to any great extent during the experiment. These data provide further evidence that the ridges observed in the SEM experiment contain the MCPA-PHBV conjugate, which gradually migrates into the soil over the course of the glasshouse experiments.

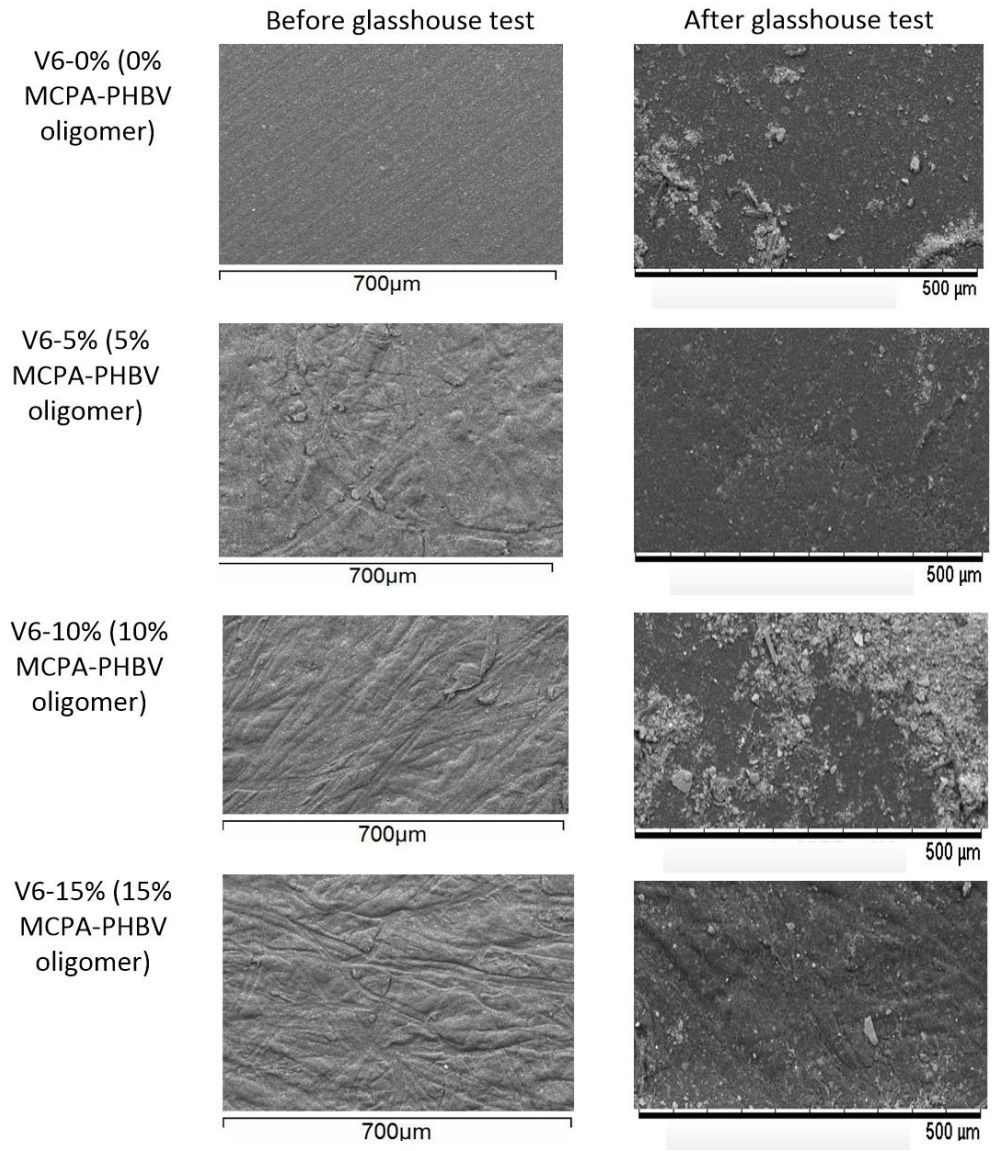

Figure 6. Scanning electron microscope images of the mulch films before and after the glasshouse experiment.

As described previously, the elemental composition of the mulch films was analysed by EDS. The percentage chlorine content of the films was identified by this analysis as this element is a 'signature' for the presence of the MCPA herbicide which is not present in the PBAT/PLA blend. The results of the analyses of the mulch films containing the herbicide are shown in Figure 7. No chlorine was detected in the control film (0% MCPA-PHBV) (data not shown).

The quantity of chlorine present in the mulch films containing the herbicide was shown to significantly decrease after four months exposure to the soil surface. In the V6-5% film over the course of the experiment the chlorine level decreased from 1.9% to 1%; in the V6-10% film it decreased from 2.1 to 0.8% and in the V6-15% film the observed decrease was from 2.7% to 1%. These data indicate that the herbicide migrated into the soil over the course of the glasshouse experiment. The MCPA is then absorbed into the tissues of the target plant species which supresses their growth. It is interesting to note that by the end of the experiment all three film samples had reached a similar chlorine level, regardless of their initial chlorine concentration.

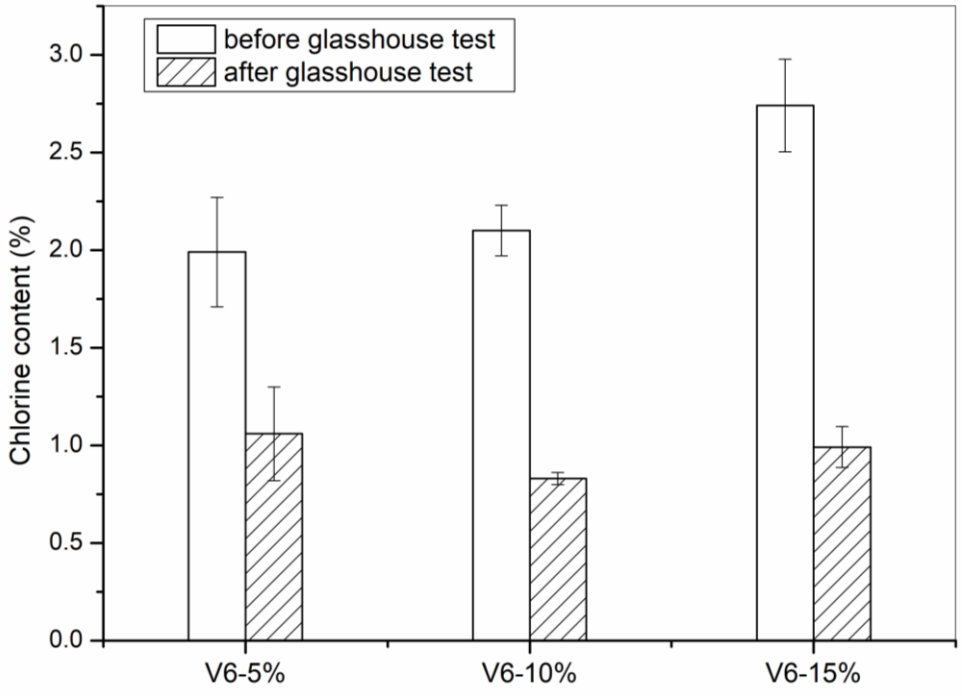

Figure 7. Chlorine content of mulch films before and after the glasshouse experiment.

Thermal properties change indicating the migration of MCPA-PHBV. In order to further understand the migration behaviour of the herbicide a DSC test of the film both before and after the glasshouse experiment was conducted, as summarized in Table 1. There are two glass

transitions in the PBAT/PLA blend, at -31.6°C and 55.2°C, which are attributed to PBAT and PLA respectively. The nearly un-shifted melting points (125 and 151 °C) and glass-transition temperatures (-31 and 55°C) of the BioFlex reference indicate the formation of a two phase system, due to the incompatibility of the constituent polymers. The sample of PHBV-MCPA conjugate shows only one glass transition temperature T_g at -12.3°C, due to the low molecular weight nature of the modified PHBV-backbone.

Table 1. DSC Thermal properties of the mulch films after the glasshouse experiment (2nd heating scan).

		T_{g1} (°C)	T_{g2} (°C)	T_{cc} (°C)	T_{m1} (°C)	ΔH_{m1} (J/g)	T_{m2} (°C)	ΔH_{m2} (J/g)
PBAT/PLA	before test	-31.6	55.2	-	125.3	2,076	150.9	1,46
	after test	-28.7	60.0	-	124.5	1,326	148.8	1,465
V6-5%	before test	-28.2	48.6	102.4	123.2	1,944	150.7	1,745
	after test	-29.4	53.3	-	124.4	3,652	-	-
V6-10%	before test	-28.0	47.9	102.2	124.1	2,482	150.7	1,745
	after test	-29.0	52.4	-	124.5	6,055	-	-
V6-15%	before test	-27.4	36.9	99.0	-	-	149.0	1,509
	after test	-29.8	51.1	-	125.4	1,81	-	
MCPA-PHBV		-12.3						

The mulch film sample V6-15% shows the corresponding T_g transitions for PBAT and PLA at -27.4°C and at 36.9°C respectively. The shifts of the T_g's of PBAT and PLA indicate that the MCPA-PHBV conjugate is miscible with PBAT and PLA and improved the mobility of the molecules in the amorphous phase. In the less enriched sample, no equivalent shift in the T_g of

PLA was seen. Furthermore, the mulch film samples show an exothermic peak at 102°C compared to the film minus the herbicide, which is attributed to the cold crystallization peak of PLA. Therefore, the bioactive conjugate may act as a nucleating agent showing a broad flat melting peak for PBAT.

However, after the glasshouse experiment, the T_g of PLA increased to 53, 52 and 51^oC, while the T_g of the sample containing no MCPA-PHBV increased to 60^oC. The increase in T_g indicates the loss of the plasticizing effect of the conjugate. The T_g shows no changes with respect to the PBAT regardless of the content of PHBV-MCPA conjugate, indicating less immiscibility of the PHBV with PBAT. This may suggest the loss of MCPA-PHBV in the samples.

Degradation of the film. Figure 8 shows the GPC traces of the films before and after the glasshouse experiment. The solid lines denote the GPC traces of the films before the experiment, and the dashed lines are the corresponding GPC traces of the films after the experiment. For the starting films, it can be seen that the higher the MCPA-PHBV content the higher is the molar mass distribution.

It is interesting to observe that all the GPC traces shift to the left compared with the starting film while there is no shift when there is no MCPA-PHBV. Moreover, the molecular mass distribution, measured using UV detector sensitive to the aromatic moieties, decreased for MCPA-PHBV containing samples. This confirms the release of MCPA-PHBV from the mulch during the glasshouse experiment, as suggested by the SEM and EDS analyses.

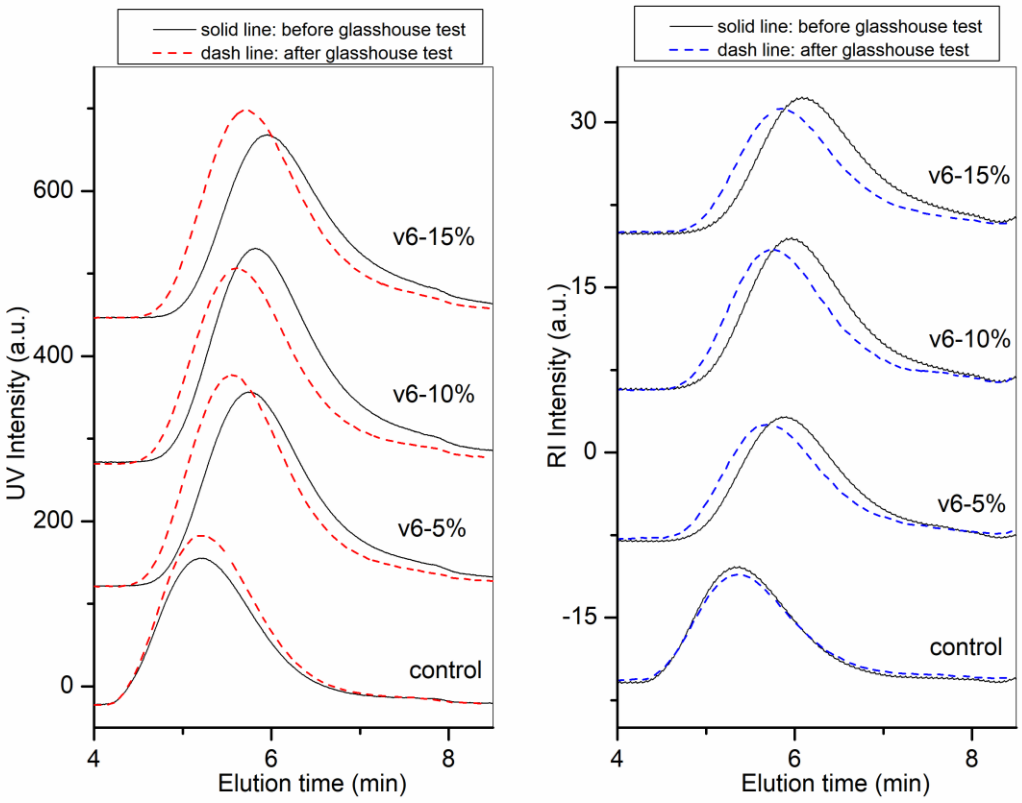

Figure 8. The GPC traces (both RI and UV detectors) of the films before and after the glasshouse experiment.

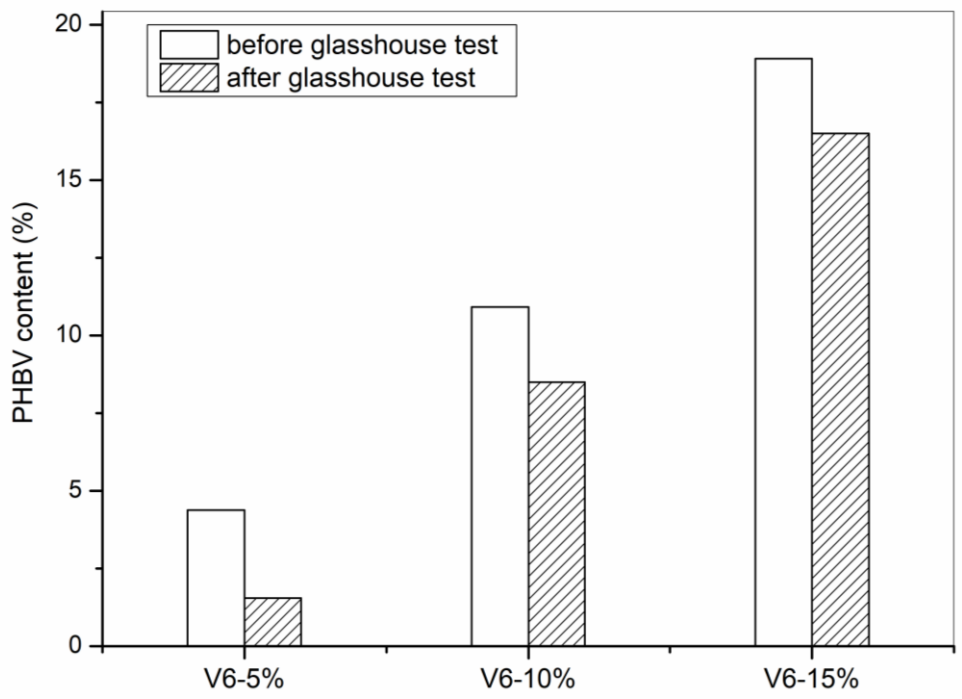

Figure 9. The composition changes of the components in the mulch films before and after the glasshouse experiment.

Figure 9 shows the changes in the PHBV content of the film recorded using NMR spectra. Both PBAT and PLA did not change significantly for various concentrations of MCPA-PHBV. However, there is a clear reduction in the PHBV content after the glasshouse experiment. It is not clear at the moment whether MCPA-PHBV diffuses out of the film as a whole or as a fragment. In order to estimate how the MCPA migrates into the soil to play a role in suppressing the growth of weeds in the glasshouse experiment, a calculation is made according to Figure 9 assuming that MCPA-PHBV diffuses out of the film as a whole without breaking down first in the film, as shown in Table 2.

Table 2. Calculation of PHBV loss based on EDS measurement of chlorine content and NMR measurement of PHBV loss after glasshouse test of the mulch film.

	V6-5% (%)	V6-10% (%)	V6-15% (%)
Chlorine loss according to EDS measurement	0.9	1.3	1.7
MCPA loss based on chlorine loss[a]	5.1	7.4	9.7
PHBV loss if no MCPA-PHBV bond scission[b]	20.6	29.7	38.8
PHBV loss according to NMR measurement	2.8	2.4	2.4

[a]The calculation is based on chlorine content in MCPA, which is 35.5/200 x 100% = 18%, where 35.5 is the atomic mass of chlorine and 200 is the molar mass of MCPA.

[b]The calculation is based on MCPA content in MCPA-PHBV oligomer, which is 200/800 x 100% = 25%, where 200 is the molar mass of MCPA, and 800 is the molar mass of the oligomer.

It can be seen from Table 2 that PHBV loss is 20.6%, 29.7% and 38.8% respectively for V6-5%, V6-10% and V6-15% if we make an assumption that MCPA-PHBV bonds do not break and that the intact molecule diffused out of the sample. However, according to NMR measurement, the loss of PHBV is 2.8%, 2.4% and 2.4% respectively. There is a large difference between the two calculations. Therefore, most of the MCPA may diffuse out of the film after bond scission. It is thus inferred that MCPA-PHBV cleaves first and then MCPA diffuses out leaving PHBV behind. Since the MCPA is conjugated with PHBV oligomer with a molar mass of around 800, a random scission of such smaller oligomeric molecules has a larger possibility to generate MCPA or MCPA conjugated with shorter PHBV chain. However, both PBAT and PLA are large molecules. Small molecules by random scission or redistribution, should be formed in a small amount. Hence, PBAT and PLA loss is insignificant, but PHBV loss is larger.

CONCLUSIONS

This study has demonstrated that the manufacture of bioactive and biodegradable PLA-PBAT based mulch films containing a MCPA-PHBV conjugate prepared by melt transesterification is feasible. The data presented has also shown that when these films are used under glasshouse conditions they are able to successfully suppress broadleaf weed species by the controlled release of the herbicide.

The glasshouse experiment demonstrated that the MCPA herbicide can diffuse out of the film, due to the presence of PHBV-MCPA oligomer, which generates small molecules containing MCPA due to random scission of the oligomer. All the analysis including GPC, SEM, EDS, DSC and NMR indicate that MCPA-PHBV diffuses out of the film, and most of the diffusing species are in a form of MCPA or MCPA conjugated with shorter PHBV chains. The GPC analysis of the mulch films over the duration of the glasshouse experiment may indicate some PLA/PBTA crosslinking phenomenon in the presence of MCPA and UV radiation although the extent of eventual crosslinking would be rather small. Though, the degradation of the mulch film over the four month duration of the glasshouse experiment is not significant. However, the PBAT/PLA blend undergoes biodegradation easily under the industrial composting conditions.[20]

The data presented has demonstrated that the MCPA-PHBV oligomer is able to supress the growth of the weed species. Moreover, at the concentrations used in this study the herbicide did not kill the (non-target) crop species. However, it did produce a mild adverse effect upon the growth/health of the faba bean plants, as judged by the measured parameters. Now that we have established the viability of the MCPA-PHBV mulch films to supress weed growth with the regulated release of a herbicide component, experiments are currently underway in our laboratory to identify the optimum concentration of MCPA-PHBV that can supress the growth of

the target weed species, without having an adverse effect on the growth of the model crop species (faba bean).

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